inases grade 2 toxicity (2 subjects) and symptoms including headache, blurred vision, abdominal pain and vomiting (3 subjects). One of these was randomized to placebo. In the 3200mg bid group, all subjects randomized to S-3304 had hepatic transaminases increased to grade 1 or 2. The correlation between plasma drug concentration and raised transaminases is yet to be determined. Only the minority of subjects reported mild myalgia/arthralgia symptoms, and this did not interfere with their normal daily activities. AUC0-12,ss and Cmax,ss of S-3304 increased with dose level but less than proportionately (see Table).

Table. Steady-State Pharmacokinetic (PK) parameters of S-3304 (mean ± SD)

Dose level	800 mg*	1600 mg*	2400 mg*	3200 mg**
$AUC_{0-12,ss}$ (μg*hr/mL)	411±117	466±80	634±170	943±317
C_{max} (μg/mL)	80±20	93±13	120±21	140±27
$T_{1/2}$ (hr)	14.2±1.2	14.8±3.4	15.9±1.8	ND

*Day 28; **Day 14 (Dose discontinued before Day 28); ND: not determined

Pharmacokinetic parameters of metabolites will also be analysed.

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Dose and schedule optimization of a novel anti-angiogenic/anti-metastatic peptide, ATN-161 (Ac-PHSCN-NH2), which targets multiple fully activated integrins including alpha-5 beta-1 and alpha-v beta-3

M.L. Plunkett[1], A.P. Mazar[1]. [1]*Attenuon, LLC, San Diego, USA;* [2]*The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA;* [3]*University of Michigan, Medical School, Ann Arbor, USA*

ATN-161 (Ac-PHSCN-NH2) is currently completing pre-clinical development with the initiation of a phase I trial anticipated in October, 2002. Previously published data has demonstrated the ability of this peptide to inhibit tumorigenesis, angiogenesis and metastasis of subcutaneously inoculated tumors in a syngeneic (Mat LyLu) model of prostate cancer [Livant et al. (2000) Cancer Res 60: 309]. In addition, ATN-161 has been shown to inhibit angiogenesis in liver metastasis from intrasplenically injected CT26 mouse carcinoma cells [Stoelzing et al., Clin Cancer Res (2001) 7: 3656s]. We have extended these results to a syngeneic Lewis Lung Carcinoma (3LL) model and have observed that ATN-161 inhibits tumor growth as effectively as metronomically administered cyclophosphamide (170 mg/kg q6d) in the early 3LL model (ATN-161 given on or before day 6 after tumor cell inoculation). We have used this 3LL model to optimize dose as well as schedule. The inhibition of 3LL tumor growth by ATN-161 observed a U-shaped dose response with 1-10 mg/kg being the optimal dose. No anti-tumor effects were observed at 0.2 mg/kg and very little effect was observed at 100 mg/kg, the highest non-toxic dose tested, with escalating anti-tumor effects observed at 50, 25 and 12.5 mg/kg, respectively. This U-shaped dose response was confirmed using the CT26 metastasis model. At the optimal dose of ATN-161 (1 mg/kg), significant inhibition of liver metastasis was observed whereas no activity was observed at the high dose of ATN-161 (100 mg/kg) tested. Schedule optimization was also evaluated using the 3LL model. The optimal schedule was determined to be ATN-161 (1 mg/kg) q3d. These results will provide rationale for the starting dose and schedule for phase I trials.

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In vitro antiangiogenic activity of thalidomide analogues

S.S.W. Ng[1], E.A. Kruger[1], F.A. Luzzio[2], K. Eger[3], M. Guetschow[3], S. Hauschildt[3], T. Hecker[3], U. Teubert[3], M. Weiss[3], W.D. Figg[1]. [1]*National Cancer Institute, Molecular Pharmacology Section, Cancer Therapeutic;* [2]*University of Kentucky, Department of Chemistry, Louisville;* [3]*Institut für Pharmazie, Universität Leipzig, Pharmazeutische Chemie, Leipzig, Germany*

Thalidomide, also known as (alpha-(N-phthalimido)-glutarimide), is currently in Phase II clinical testing as a single agent or in combination with chemotherapy against a number of solid tumors such as gliomas, prostate and renal cell carcinomas. The resurgence of interest in thalidomide can be attributed to its antiangiogenic activity, which was shown to be mediated by a metabolilte – 5'OH thalidomide. Using the backbone of the metabolite, we synthesized 118 unique thalidomide analogues and examined their antiangiogenic activity *in vitro*. Preliminary experimental data selected seven of these analogues for further evaluation. In the rat aortic ring assay, six of the seven analogues significantly inhibited microvessel outgrowth at 12.5-200 uM. Thalidomide failed to block angiogenesis at similar concentrations. Subsequently, the effects of these analogues on human umbilical vein endothelial cell (HUVEC) proliferation and tube formation were studied. Six of the seven analogues demonstrated antiproliferative action in HUVECs. Cell proliferation was not affected by thalidomide. Interestingly, all seven analogues as well as thalidomide suppressed tube formation. Analogues in the tetrafluorophthalimido class showed the highest potency and efficacy in all three assays. Taken together, our results support the further development of thalidomide analogues as antiangiogenic agents. The *in vivo* toxicology and therapeutic potential of the described analogues in the treatment of prostate cancer are presently under investigation.

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VEGF-Trap: a novel, potent VEGF blocker with anti-tumor effects

J. Holash, J. Rudge, S. Davis, N. Papadopoulos, S. Wiegand, G. Yancopoulos, J. Cedarbaum. *Regeneron Pharmaceuticals, Inc., Tarrytown, USA*

Vascular endothelial growth factor (VEGF) plays a critical role during the normal process of angiogenesis required for embryonic development, and plays a key role in the pathological angiogenesis that occurs in a number of diseases, including cancer. One of the most effective ways to block the VEGF-signaling pathway is to prevent VEGF from binding to its normal receptors by administering decoy soluble receptors. By determining the requirements to maintain high affinity while extending *in vivo* half-life, we were able to engineer a very potent VEGF blocker with desirable pharmacokinetic properties. The resulting VEGF Trap, a soluble decoy receptor created by fusing the ligand-binding immunoglobulin domains of VEGF receptor 1 (VEGFR1) and VEGF receptor 2 (VEGFR2) to the constant region (Fc) of human IgG1, is the highest affinity VEGF blocker described to date, with an affinity for VEGF of 1-5 pM. The VEGF-Trap effectively suppresses tumor growth and vascularization *in vivo*, resulting in stunted, and almost completely avascular tumors. VEGF Trap mediated blockade may be superior to that achieved by other agents, such as monoclonal antibodies targeted against the VEGF receptor. The VEGF Trap is currently undergoing a Phase I clinical trial.

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Tumor genotype, RRM1 expression, and outcome of patients with lung cancer

G. Bepler, S. Sharma, A. Gautam, P. Smith, Z. Zheng, J. Hofmann, G. Simon. *H. Lee Moffitt Cancer Center and Research Institut, Thoracic Oncology Program, Tampa, USA*

We have described frequent allele loss on chromosome segment 11p15.5 and its association with metastatic spread and shortened survival in patients with non-small cell lung cancer. Patients with stage I disease, i.e. absence of spread to lymph nodes, and allele loss had survival comparable to patients with stage II disease, i.e. cancer present in lymph nodes. We have recently reported the complete genomic sequence and transcript map for the minimal region of allele loss, and it encompasses the complete gene for the regulatory subunit of ribonucleotide reductase (RRM1). However, functional inactivating mutations were not found by screening a subset of NSCLC. Our recent cell biological studies have provided evidence for a functional role of RRM1 in suppression of cell migration, invasion, and *in vivo* metastasis formation, that is independent of an alteration in the deoxynucleotide (dNTP) pool. Other investigators have reported reduced anchorage independent growth in ras-transformed mouse fibroblasts transfected with RRM1 and a role for RRM1 in microtubule nucleation of centromeres in Xenopus. Here, we measured the level of expression of RRM1, compared to the expression of RRM2 (catalytic subunit of ribonucleotide reductase) and p53R2 (catalytic subunit involved in dNTP supply for DNA damage repair), and investigated the association with allele loss, RRM1 promoter polymorphisms, and survival. Tissue specimens from 51 patients undergoing resection for NSCLC were collected and immediately frozen in liquid nitrogen. Total RNA was extracted, reverse transcribed, and used for real-time quantitative PCR (ABI Prism 7700). Primers and probes for the genes RRM1, RRM2, and p53R2 were designed to cross introns, and the amplicons were 95 bp, 90 bp, and 105 bp respectively. Gene expression was normalized using 18S rRNA as reference. We found that RRM1 expression was associated with RRM1/D11S4932 allele loss, with a median RRM1 value of 3.8 in specimens with allele loss compared to 43.7 in those without allele loss. RRM1 expression was also associated with the A/C promoter polymorphism, with a median RRM1 value of 12.9 in patients with the CC allelotype, 72.8 in those with the AA allelotype, and 22.8 in heterozygotes (AC allelotype). RRM1 and RRM2 expression were highly correlated